

FINAL Report

5R01HL088886 – Neuronal Determinants of Respiratory Rhythmogenesis

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Specific Aims. No changes since original proposal.

Studies and Results. This project is focused on experimental data analysis and the development of computational models of the network of neurons in the mammalian brainstem that generate and control the respiratory rhythm. Our efforts are largely focused on understanding a particular component of this complex neuronal circuitry: the pre-Bötzinger Complex (pBC). The pBC is believed to be a major contributor to the inspiratory phase of the respiratory rhythm, and this region can be contained in a transverse brain-slice through the medulla. The pBC slice contains both the pBC as well as transmission circuitry to the hypoglossal (XII) nucleus. This *in vitro* slice is rhythmically active, outputting a regular pattern of electrical activity that is believed (*in vivo*) to contribute towards the inspiratory phase of the respiratory rhythm.

Since the last progress report in early 2011, one postdoctoral fellow was supported on the project.

Project: Two types of independent bursting mechanisms in inspiratory neuron: an integrative model

The network of coupled neurons in the pBC of the medulla generates a bursting rhythm, which underlies the inspiratory phase of respiration. In some of these neurons, bursting persists even when synaptic coupling in the network is blocked and respiratory rhythmic discharge stops. Bursting in inspiratory neurons has been extensively studied, and two classes of bursting neurons have been identified, with bursting mechanism depends on either persistent sodium current or changes in intracellular Ca^{2+} , respectively. Motivated by experimental evidence from these intrinsically bursting neurons, we present a two-compartment mathematical model of an isolated pBC neuron with two independent bursting mechanisms. Bursting in the somatic compartment is modeled via inactivation of a persistent sodium current, whereas bursting in the dendritic compartment relies on Ca^{2+} oscillations, which are determined by the neuromodulatory tone (Figure 1). Depending on a parameter choice, the model produce persistent sodium (somatic) bursting, Ca^{2+} -based (dendritic) bursting or mixed (somato-dendritic) bursting.

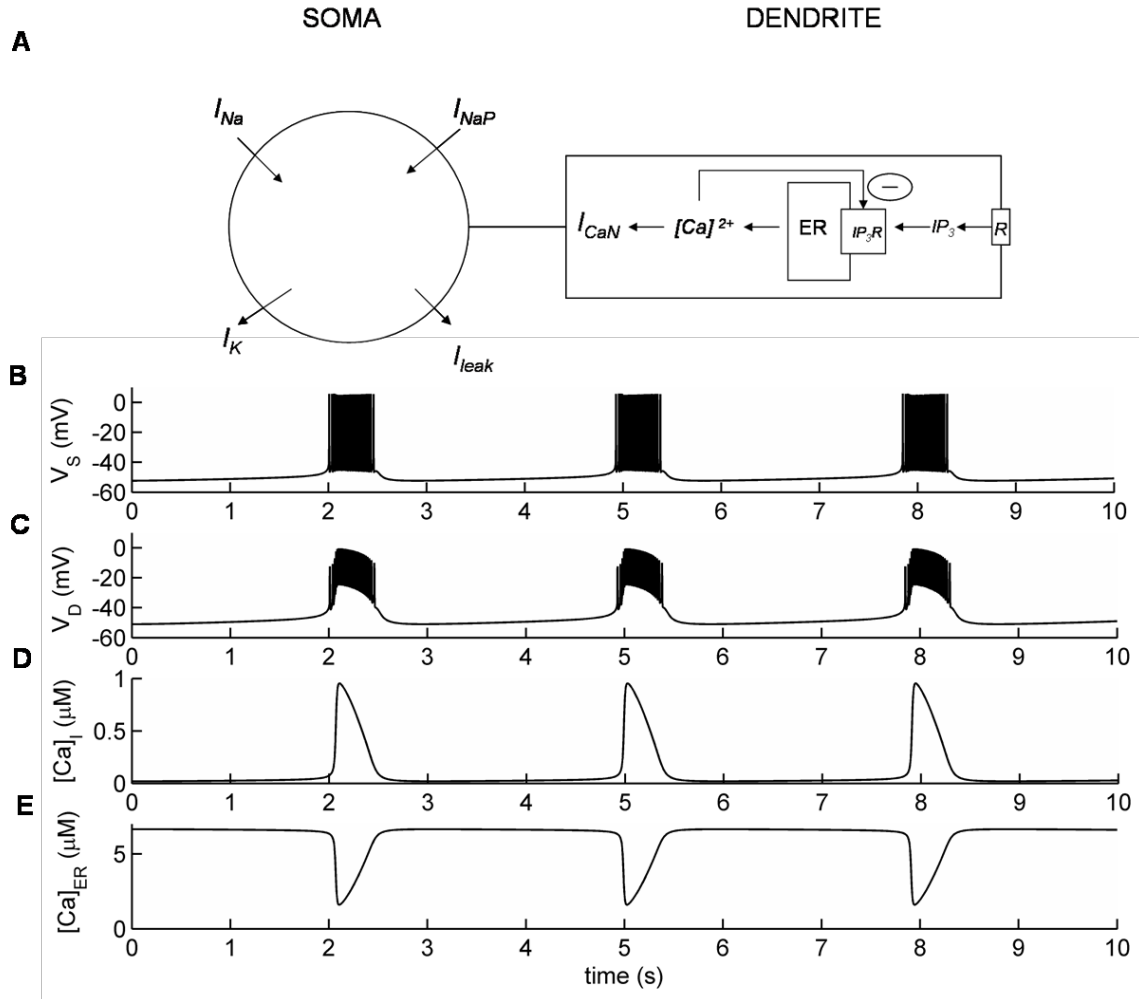


Figure 1. Illustration of the dynamics of the model (A) Sketch of the model. (B) Voltage in soma. (C) Voltage in the dendrite. (D) Intracellular Ca^{2+} concentration in the dendrite. (E) Dendritic ER Ca^{2+} concentration.

The model explains a number of conflicting experimental results and is able to generate a robust bursting rhythm, over a large range of parameters, with a frequency adjusted by neuromodulators. It has been reported that INaP- and ICaN- subpopulations of inspiratory intrinsic bursters in the mouse brainstem has different response to several pharmacological blockers, which affect either persistent sodium current or Ca^{2+} currents. Our model can reproduce these conflicting results. Cadmium, a non-specific blocker of Ca^{2+} channels, can suppress burst in dendritic bursters (Fig 2C, middle), while the other subpopulations (somatic and somato-dendritic) remained active (Fig 2A,B, middle). In response to a specific blocker of INaP, Riluzole (RIL), somatic bursting stops (Fig. 2A, right), but somato-dendritic and dendritic bursters remain rhythmic but fire less spikes (Fig. 2B,C, right).

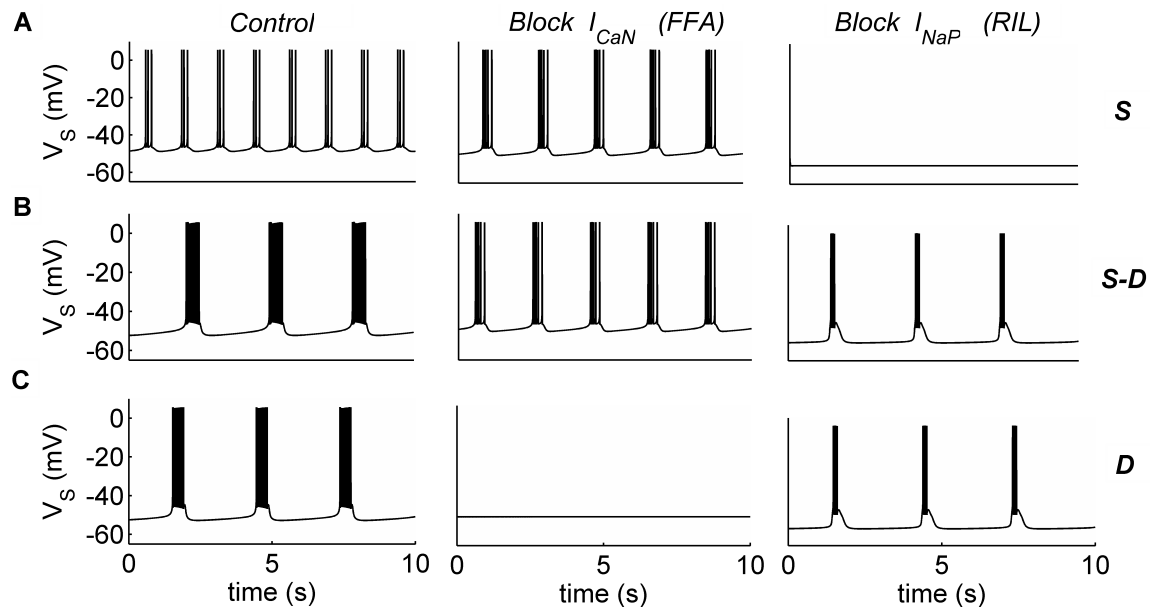


Figure 2. Effect of I_{CaN} and I_{NaP} blockers on different types of bursters. (A) A somatic burster (S) is blocked by the I_{NaP} blocker RIL (right), but persists under the I_{CaN} blocker FFA (middle). (B) A somato-dendritic burster (S-D) persists under the both I_{NaP} and I_{CaN} blockers. (C) A dendritic burster (D) is blocked by the I_{CaN} blocker FFA (middle), but persists under the I_{NaP} blocker RIL (right).

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Project: Conflicting effects of excitatory synaptic and electric coupling on the dynamics of square-wave bursters

Using two-cell and 50-cell networks of square-wave bursters, we studied how excitatory coupling of individual neurons affects the bursting output of the network. Our results show that the effects of synaptic excitation vs. electrical coupling are distinct. Increasing excitatory synaptic coupling generally increases the burst duration (Figure 3C). Electrical coupling increases burst duration for low to moderate values, but at sufficiently strong values promotes a switch to highly synchronous bursts where further increases in electrical or synaptic coupling have a minimal effect on the burst duration (Figure 3B). These effects are largely mediated by spike synchrony, which is determined by the stability of the in-phase spiking solution during the burst (Figure 3 E,F)). Even when both coupling mechanisms are strong, one form (in-phase or anti-phase) of spike synchrony will determine the burst dynamics, resulting in a sharp boundary in the space of the coupling parameters (Figure 3 A,D). This boundary exists in both two cell and network simulations. We use these results to interpret the effects of gap-junction blockers on the neuronal circuitry that underlies respiration.

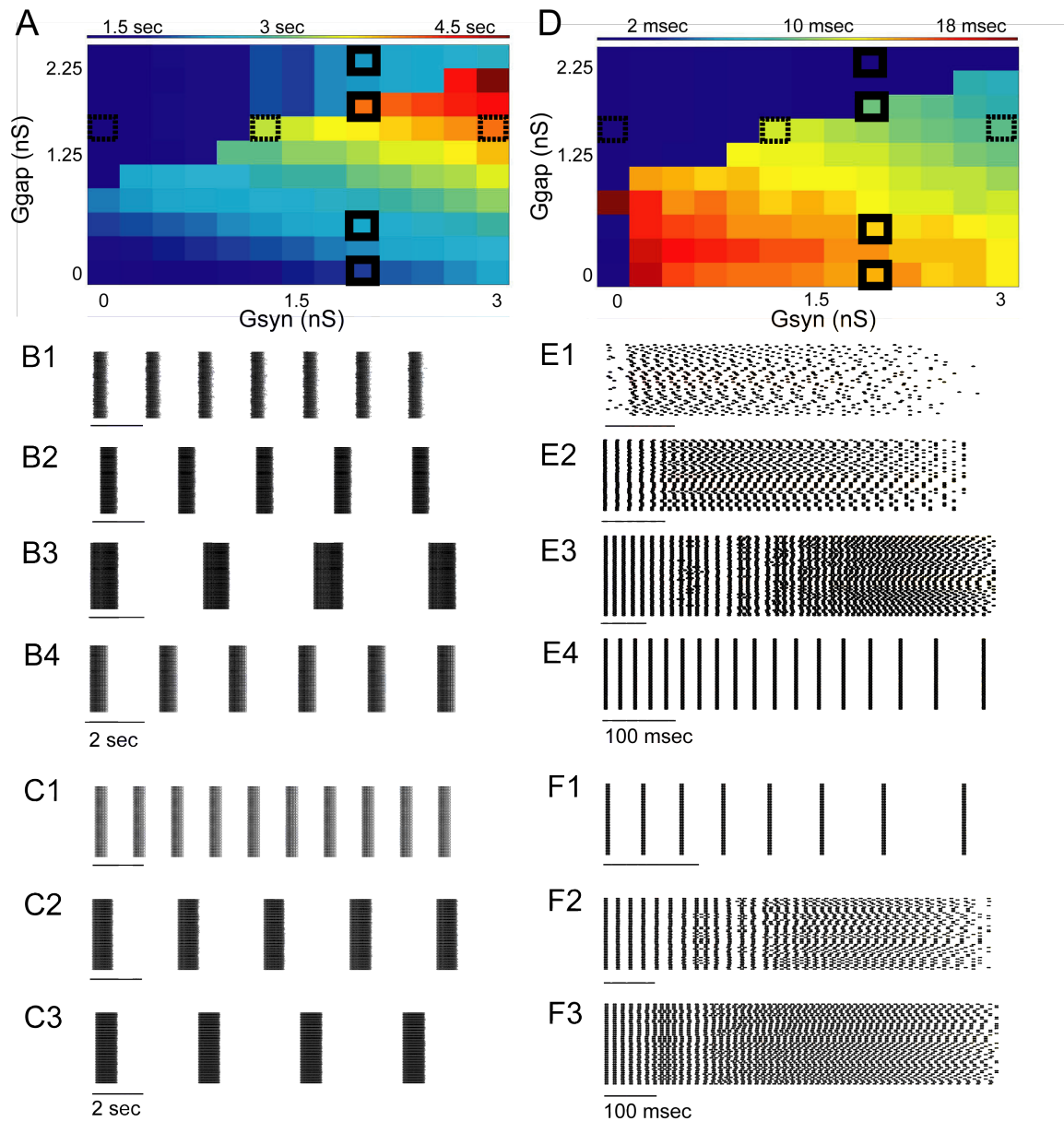


Figure 3. Effects G_{gap} and G_{syn} on burst period (panel A) and spike synchrony (panel D). The Spike synchrony is color-coded in panel D, with dark blue color representing full synchrony. Panels B/E are raster plots of several periods of bursting (B) and a single burst (E) within the network for 4 sets of coupling parameters indicated by the solid black boxes in panels A/D; the parameter sets are: $G_{gap} = 0, 1, 2,$ and 2.25 nS, with G_{syn} fixed at 2.25 nS. Panel C/F contain the same information for another 4 sets of coupling parameters indicated by the

dashed black boxes in panels A/D; these parameters are: $G_{gap} = 1.5 \text{ nS}$, with $G_{gap} = 0, 1.25$, and 3 nS .

Project: Mathematical model for frequency modulation in the respiratory network

Neuromodulators, such as amines and neuropeptides, alter the activity of neurons and neuronal networks. In this work, we investigate how neuromodulators which activate G-proteins and second messenger systems can modulate the frequency of bursting neurons in the pBC. Inspiratory neurons in the pBC produce a regular bursting rhythm in phase with the activity of inspiratory muscles in the diaphragm. These neurons are a vital part of the ponto-medullary neuronal network, which generates a stable respiratory rhythm. The frequency of pBC depends on the concentration of Serotonin (5-HT) and Substance P (SP), neurotransmitters released by the nearby Raphe nucleus. Both neurotransmitters, 5-HT and SP, affect pBC neurons by activating receptors coupled with the Gq protein pathway, thereby inducing Ca^{2+} release from the Endoplasmic Reticulum (ER).

We have previously developed a mathematical model of the pBC neuron (described in first project above), which incorporates explicit activation of Gq-protein coupled receptors, and have shown that activation of these receptors can result in Ca^{2+} oscillations in the dendritic compartment. The model exhibits two independent bursting mechanisms – bursting in the soma depends on persistent sodium current, whereas bursting in the dendrite follows Ca^{2+} oscillations. It has been recently found that the connection between the pBC and the Raphe nucleus is bi-directional: not only does the Raphe nucleus release 5-HT and SP to modulate the frequency of pBC neurons, but also the rhythmic activity in the pBC increases the firing of Raphe neurons. In this work, we extend our model to a network of pBC neurons while incorporating this newly discovered interaction between Raphe and pBC nuclei.

Using a simulated 50-cell network of excitatory connected pBC neurons with a heterogeneous distribution of persistent sodium conductance and ER Ca^{2+} , we show that a tonic release of neurotransmitters acting on the Gq protein pathway increases the number of intrinsic bursters in such a network. However, when we simulated the application of different concentrations of SP or 5-HT, there was no dose-dependent frequency modulation. We then added a positive feedback between the Raphe excitability and pBC activity, representing the release of neurotransmitters from Raphe, and found that this feedback induces frequency modulation the pBC neurons (Figure 4). Thus, our model shows that the frequency of the respiratory rhythm can be modulated via phasic release of 5-HT and SP from the Raphe nucleus.

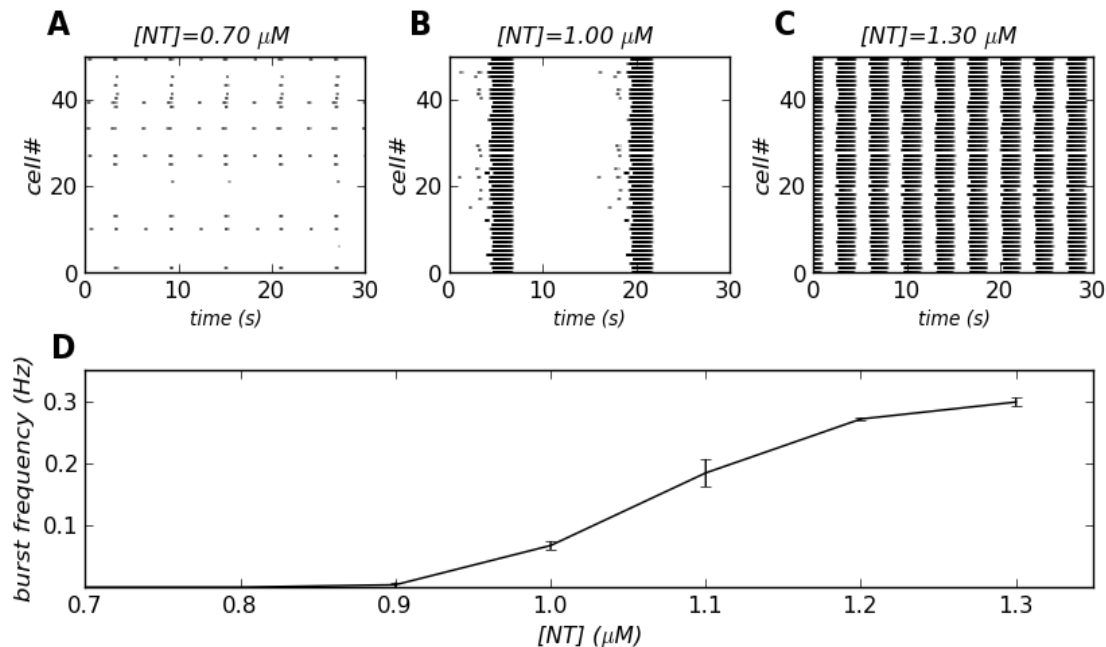


Figure 4. Inspiratory frequency modulation of pBC by excitatory neurotransmitters, which act on Gq-coupled receptor. $[NT]$ represents neurotransmitter concentration. (A-C) Example of raster plots for three different neurotransmitter concentrations. (A) Rhythmic activity is absent for low concentration of neurotransmitter ($[NT] = 0.7 \mu M$). (B) Increase in neuromodulatory tone ($[NT] = 1 \mu M$) results in a slow bursting rhythm. (C) Elevation of neurotransmitter concentration ($[NT] = 1.3 \mu M$) increases the burst frequency in pBC. (D) Burst frequency of pBC neurons as a function of neurotransmitter concentration. The vertical bars represent the standard error from an average of 10 network simulations.

Project: Methods of synchronization among non-identical neurons.

A key fundamental issue in computational neuroscience is understanding how cellular and synaptic properties enable the synchronization of firing in a population of neurons, and the nature of that synchronization (e.g., in-phase, firing simultaneous; anti-phase or “taking turn”). These ideas are fundamental to understanding rhythm-generating circuits such as the pBC. Many methods have been developed in the last 20 years to analyze models to identify the mechanisms through which these models synchronize when coupled into populations, and these results have been experimentally verified in some cases. For example, it is now commonly accepted that under appropriate conditions mutual inhibition in a network can give rise to in-phase synchrony, a finding initially considered counter-intuitive 20 years ago. However, most of these analyses do not consider the fact that neurons are typically not identical, and pose considerable cell-to-cell variability. In this study, we are extending previous

analyses (vanVreeswijk et al., 1994) commonly used to study synchronization to consider the effects of variability in properties of the coupled cells.

Figure 5 below shows a typical 2 cell simulation, with two cells coupling by synapses of identical strength.

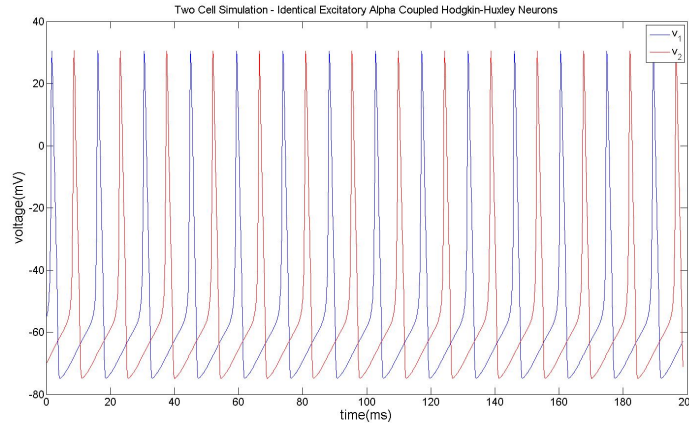


Figure 5: Typical 2 cell spiking neuron stimulation with a constant phase difference between neurons.

In this case the cells and the coupling strength are identical. The relationship in spike times is often called the “phase difference”, and represents the fraction into the cycle of one cell where the next cell fires. In the above example, the phase difference is 0.5 (this is called “anti-phase” by many investigators).

In practice, a significant degree of heterogeneity in synaptic conductance is present in neuron to neuron connections. We studied the dynamics of weakly coupled pairs of neurons with heterogeneities in synaptic conductance using Wang–Buzsaki and Hodgkin–Huxley model neurons with different classes of excitability. This type of heterogeneity breaks a symmetry in the bifurcation diagrams of equilibrium phase difference versus the synaptic rate constant when compared to the identical case. For weakly coupled neurons coupled with identical values of synaptic conductance a phase locked solution exists for all values of the synaptic rate constant, α . In particular, in-phase and anti-phase solutions are guaranteed to exist for all α . Heterogeneity in synaptic conductance results in regions where no phase locked solution exists and the general loss of the ubiquitous in-phase and anti-phase solutions of the identically coupled case. We explain these results through examination of interaction functions using the weak coupling approximation and an in-depth analysis of the underlying multiple cusp bifurcation structure of the systems of coupled neurons.

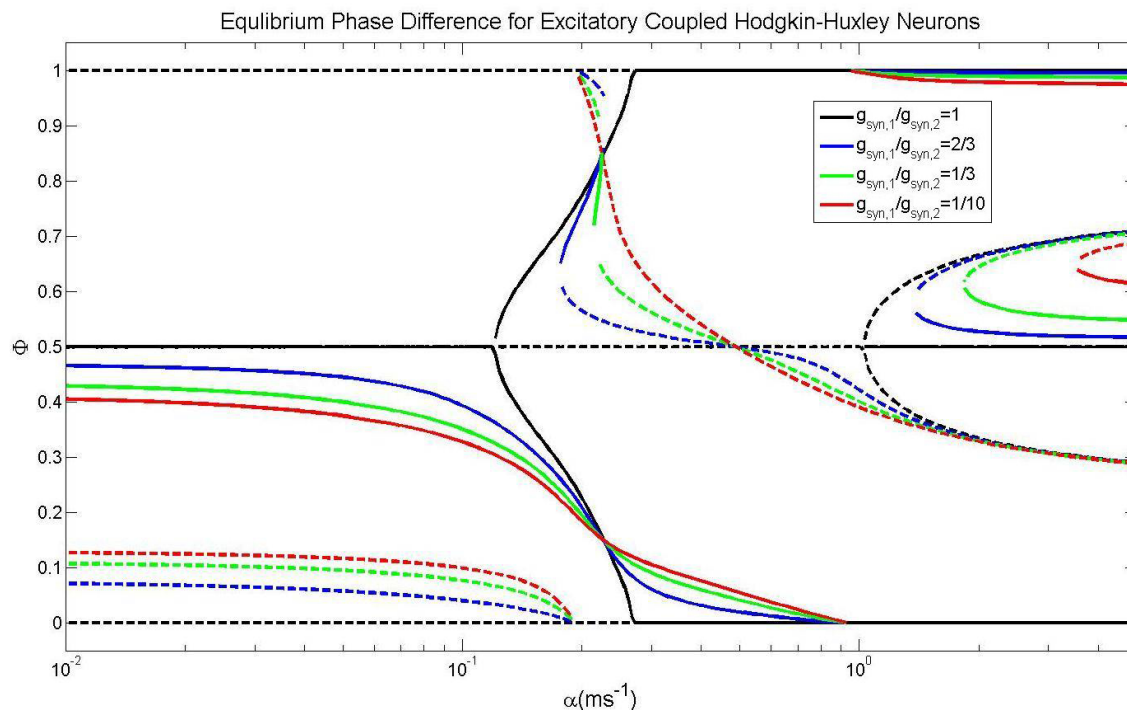


Figure 6. *Equilibrium phase difference between two coupled neurons as a function of synaptic rate constant (X axis) and degree of dissimilarity in reciprocal conductances (see legend).*

Figure 6 plots the phase difference between two neurons as a function of the synaptic rate constant (x axis). The black traces are for identical cells, and represent commonly accepted and previously published results. The colored traces indicate how this diagram is perturbed asymmetrically by non-uniform coupling between the two cells. Solid lines represent stable long-term phase relationships.

The take-home message from these results is that if neurons are non-identical, one should not be looking simply for in-phase and anti-phase relationships, but that other stable relationships are possible. Furthermore, when the neurons are non-identical, there exist some synaptic rate constants where synchrony is not possible. We intend to apply these results to study not only neural dynamics within the pBC, but also at a macroscopic level to study the relationship between pBC and pFRG rhythms, which we previously published in a conference paper.

Status: Published in the *Journal of Computational Neuroscience* in late 2011. PMC3059364.

Project: Evaluation of Poon's "handshake" model for pBC/pFRG interactions

In Wittmeier, Song, Duffin and Poon (PNAS 2008), the authors generate quantal slowing using a two cell model of the respiratory rhythm circuit; this model contains the pBC and the prefrontal respiratory group (pFRG). Each cell possesses a persistent sodium current, a fast sodium current, a potassium current, and a leak current. Synaptic connections from the pFRG to the pBC are excitatory while connections from the pBC to the pFRG are inhibitory. An inhibitory current to the pBC is active during the rebound burst of the pFRG; this off-switch current models inhibitory feedback. The inhibitory synapse increases by 1 nS with each presynaptic spike and decays with a time constant of 25 ms. The excitatory synapse is stochastic; a new conductance is selected from a Gaussian distribution with each presynaptic spike, and negative conductance values are set to zero. The standard deviation (sigma) of the normal distribution (called the variance by the authors) is set to one half the mean (mu) and the default normal parameters at which no quantal slowing is exhibited are $\mu=0.120$ nS and $\sigma=0.06$ nS. As the mean and standard deviation are decreased, more pBC bursts are skipped. At $\mu=0.06$ nS and $\sigma=0.030$ nS (50% of default), an interburst interval histogram reveals that the primary pattern exhibited shifts from 0 bursts skipped to one burst skipped.

Wittmeier and colleagues use only mus and sigmas 50-100% of the default in their publication. As we decreased mu and sigma to 40% of default and below, more bursts were skipped and interburst intervals became longer. Because simulations were run for a set time, more skipped intervals resulted in fewer interburst intervals plotted on the histograms. At 10% of the default mean and standard deviation, no pBC bursts were recorded at all during the simulation time. The Gaussian shape was altered by varying mu and sigma separately; when sigma, or Gaussian width, was held constant, a decrease in the mean value resulted in more quantal slowing. If mu was held constant, an increase in sigma decreased the incidence of quantal slowing for the low mean values and did not affect quantal slowing at high mean values. The shape of the Gaussian appears to influence when quantal slowing occurs, but the general trend of more quantal slowing as the Gaussian is shifted down and made narrow is preserved. To make the model more representative of a network, we altered it by adding either a continuously changing excitatory synapse or adding an exponential decay to the synapse. For the continuously changing g_{synE} , a value was drawn from a normal distribution at each integration time step as opposed to at each presynaptic event; this assumes more random background activity than event-based changing. Using the continuously selected conductance, the same general trend is observed; quantal slowing is more frequent as mean and standard deviation decrease. The mu and sigma where skipping one burst becomes the primary mode of operation occurs at a higher value than in the normal Wittmeier model case. At 50% of the default mu and sigma, more than one burst is always being skipped by the pBC. The second alteration to increase network realism involved selecting a new stochastic conductance with every presynaptic event and adding an exponential decay to it. The exponential decay model could produce quantal slowing at the default values of mu and sigma, something that

was not seen in the normal Wittmeier model. As mean and standard deviation were decreased, quantal slowing became more evident and multiple pBC bursts were skipped regularly.

In summary, the Wittmeier model is not as representative of network behavior as it could be. Some of its components, like the off switch current and the stochastic synapse, may be difficult to justify. It is a two cell model that attempts to represent two networks of multiple cells. However, for all these shortcomings, the simple two cell model can still describe the general trend that is predicted by using more network-representative two cell networks, which is that as the μ and σ of the excitatory conductance gets smaller, the incidence of quantal slowing increases. This explanation is also intuitive, as it suggests that quantal slowing is the result of weaker synaptic excitation. The stochastic synapse is necessary for quantal slowing in this model; if it is taken out and the model made deterministic, no pBC bursting occurs at the default conductance values used in the paper. It is only when the conductance is greater than or equal to 0.16 nS that bursting in the pBC starts, and it is in a regular pattern with the pFRG, as there are no obvious skips. This also suggests a certain level of excitation is necessary for regular bursting, and this level can be reached by having a stochastic synapse with a large enough mean or a wide enough standard deviation.

Status: We concluded that while the published model by Wittmeier et al. (2008) has flaws, even with correcting those flaws the general qualitative mechanisms proposed by the author are still valid, thus we have stopped work on this effort.